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Evaluation of lymphocyte crossmatch (CDCXM and Flow XM) test results before kidney transplantation

Böbrek nakli öncesi yapılan lenfosit crossmatch (CDCXM ve Flow XM) test sonuçlarının değerlendirilmesi

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Abstract

Purpose: Detection of anti-HLA antibodies is crucial in pre-transplant immunological assessment to evaluate donor-recipient compatibility and prevent hyperacute rejection. This study aimed to evaluate lymphocyte crossmatch (XM) test results performed before kidney transplantation.

Materials and methods: Lymphocyte crossmatch tests conducted at the Tissue Typing Laboratory of Pamukkale University Hospital between February 2020 and February 2024 were retrospectively analyzed. CDCXM (Complement-Dependent Cytotoxicity Crossmatch) and flow cytometric crossmatch (FCXM) tests were performed. For CDCXM, T and B lymphocytes were separated and tested individually, with serial dilution and DTT treatment applied. FCXM used anti-CD3 for T lymphocytes, anti-CD19 and anti-IgG for B lymphocytes, analyzed on a 10-color, 3-laser flow cytometer (Navios EX-Beckman Coulter, Inc. USA).

Results: A total of 225 CDCXM test results, 105 of which were alive and 120 of which were cadaver, were included in the study. CDCXM T and FCXM T were negative in living donors, while CDCXM B and FCXM B positivity were 4.8% and 2.6%, respectively. CDCXM T and B positivity in cadaveric donors were 3.3% and 7.5%, respectively, and FCXM T and B positivity were 3.3% and 8.8%, respectively.

Conclusion: Lymphocyte crossmatch remains a reliable and classical method for pre-transplant immunological evaluation. It effectively detects donor-specific anti-HLA antibodies and contributes to the prevention of hyperacute rejection in kidney transplantation.

Keywords: Kidney transplantation, lymphocyte crossmatch, CDC crossmatch, flowcytometric crossmatch, anti-HLA antibodies.

Mete E. Evaluation of lymphocyte crossmatch (CDCXM and Flow XM) test results before kidney transplantation. Pam Med J 2026;19:423-428.

Öz

Amaç: Anti-HLA antikorlarının tespiti, donör-alıcı uyumunu değerlendirmek ve hiperakut reddi önlemek için nakil öncesi immünolojik değerlendirmede kritik öneme sahiptir. Bu çalışmada, böbrek nakli öncesinde yapılan lenfosit çapraz uyum (XM) testi sonuçlarının değerlendirilmesi amaçlanmıştır.

Gereç ve yöntem: Şubat 2020 ile Şubat 2024 tarihleri arasında Pamukkale Üniversitesi Hastanesi Doku Tipleme Laboratuvarı'nda gerçekleştirilen lenfosit çapraz uyum testleri retrospektif olarak analiz edilmiştir. CDCXM (Komplement Bağımlı Sitotoksisite Çapraz Uyum) ve akım sitometrik çapraz uyum (FCXM) testleri gerçekleştirilmiştir. CDCXM için T ve B lenfositleri ayrı ayrı ayrı test edilmiş, seri dilüsyon ve DTT uygulanmıştır. FCXM'de T lenfositleri için anti-CD3, B lenfositleri için anti-CD19 ve anti-IgG kullanılmış ve 10 renkli, 3 lazerli akım sitometresinde (Navios EX-Beckman Coulter, Inc. USA) analiz edilmiştir.

Bulgular: Çalışmaya 105'i canlı ve 120'si kadavrada olmak üzere toplam Toplam 225 CDCXM test sonucu dahil edilmiştir. Canlı donörlerde CDCXM T ve FCXM T negatif bulunurken, CDCXM B ve FCXM B pozitifliği sırasıyla %4,8 ve %2,6 idi. Kadavra donörlerde CDCXM T ve B pozitifliği sırasıyla %3,3 ve %7,5, FCXM T ve B pozitifliği ise sırasıyla %3,3 ve %8,8 idi.

Sonuç: Lenfosit çapraz eşleştirme, nakil öncesi immünolojik değerlendirme için güvenilir ve klasik bir yöntem olmaya devam etmektedir. Vericiye özgü anti-HLA antikorlarını etkili bir şekilde tespit eder ve böbrek naklinde hiperakut reddin önlenmesine katkıda bulunur.

Anahtar kelimeler: Böbrek nakli, lenfosit çapraz eşleştirme, CDC çapraz eşleştirme, flowsitometrik çapraz eşleştirme, anti-HLA antikorları.

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Introduction

Kidney transplantation is the preferred treatment method for patients with end-stage renal disease or severe chronic kidney disease due to its ability to improve quality of life and offer better survival advantages compared to dialysis [1-3].

The human leukocyte antigen (HLA) system plays an important role in renal graft acceptance. Longer and better graft survival has been reported in patients with HLA-identical siblings and non-reactive cytotoxicity assays (CDCXM). New HLA typing methods and anti-HLA antibody detection techniques, such as flow cytometry, solid-phase immunological tests, or antigen bead assays, have further improved outcomes for kidney transplant recipients [4].

Anti-human leukocyte antigen (HLA) antibodies in the serum of patients requiring organ transplantation are a significant risk factor [5]. To detect anti-HLA antibodies, Terasaki and Mc Clelland defined a serological method in 1964 called the microlymphocytotoxicity method, also known as the complement-dependent cytotoxicity (CDC) test [6, 7].

The CDC Crossmatch test (CDCXM) is used to detect antibodies developed against donor HLA antigens. It is a test system in which the patient's serum is compared with the donor's lymphocytes. It must be demonstrated that the recipient's serum does not already contain antibodies against the donor's lymphocytes. If transplantation is performed when the crossmatch is positive, it may cause hyperacute rejection [8].

T-cell CDCXM: T lymphocytes express only class I HLA molecules, and a positive T-cell crossmatch test can lead to very poor outcomes [9]. Patel and Terasaki examined the results in 30 transplant recipients with positive T-cell crossmatch tests and reported immediate graft loss in 24 patients and early graft rejection within 3 months in the remaining patients [10].

B-cell CDCXM: B lymphocytes express both class I and class II HLA molecules. B-cell crossmatch detects antibodies to class II HLA molecules. However, positive results are not as definitive as positive T-cell crossmatches due to a higher rate of false-positive results (50%) and time constraints in the case of organ transplant failure [11]. Negative results are reassuring, meaning that even if the T-cell crossmatch test is positive, it will be due solely to non-HLA antibodies [9].

FCXM Crossmatch testing is also performed using flow cytometry. CDCXM and FCXM tests can be performed separately by separating T and B lymphocytes. Like CDCXM, the FCXM test involves incubating donor cells and recipient serum, but complement is not added; instead, a fluorescently labeled anti-IgG antibody is used to detect the presence of donor-specific IgG antibodies that bind to lymphocyte surface antigens such as HLA. The additional use of anti-CD3 and anti-CD19 or CD20 monoclonal antibodies allows the distinction of T and B lymphocyte subpopulations, respectively. The FCXM test is analyzed using a Flow Cytometer, allowing us to compare the mean fluorescence intensity (MFI) results emitted by fluorescent dye-labeled anti-human IgG bound to donor cells incubated with recipient serum with the MFI of the same cells treated with negative control serum [12]. Donor-specific antibodies (DSA) detected by crossmatch tests (especially CDCXM T) should be considered a contraindication to transplantation. This can prevent hyperacute rejection and immediate graft loss [9, 10, 13].

This study aimed to evaluate the results of the lymphocyte crossmatch test performed before kidney transplantation at the Pamukkale University Hospitals Tissue Typing Laboratory between February 2020 and February 2024.

Materials and methods

This study evaluated the results of the lymphocyte crossmatch test performed at the Pamukkale University Hospitals Tissue Typing Laboratory between February 2020 and February 2024. Lymphocyte crossmatch tests were performed using CDCXM and FCXM.

The CDCXM test was performed separately by separating T and B lymphocytes into CDCXM T and CDCXM B. The CDCXM T and B tests were also performed with serial dilutions. In addition, dithiothreitol (DTT) treatment was applied to selected samples to differentiate

between IgM and IgG antibodies. DTT reduces disulfide bonds in IgM molecules, thereby eliminating IgM-mediated cytotoxicity while preserving IgG antibody activity. Comparison of results obtained before and after DTT treatment allowed the identification of the immunoglobulin class responsible for the observed cytotoxicity. Additionally, an autocrossmatch test was performed to investigate the presence of autoantibodies [14-16].

For the FCXM test, T and B cells separated using a magnetic method were transferred to another tube as a 25 µl cell suspension and distributed into three tubes for negative and positive controls to perform crossmatching. Subsequently, 25 µl of control serum and patient serum were added to each tube and incubated for 30 minutes at room temperature. After incubation, 1 ml of isotonic solution was added and the samples were centrifuged at 1900 rpm. This washing step was repeated three times under the same conditions.

To identify T and B lymphocytes, 5 µl of CD3-PerCP and to identify B lymphocytes, anti-CD19 (CD19-PE) (PE: phycoerythrin) (BD, CA, USA) monoclonal antibodies were added. In addition, 50 µl of human anti-IgG FITC (BioLegent, CA, USA) secondary antibody was added to detect bound IgG antibodies. Following a further incubation for 30 minutes at room temperature, the cells were washed twice. Finally, 500 µl of isotonic solution was added to each tube, and the samples were analyzed using a Beckman Coulter Navios EX flow cytometer (Beckman Coulter, Inc., USA) [17].

Permission was obtained from the Pamukkale University Non-Interventional Clinical Research Ethics Committee for the study (permission date:26.11.2025, permission number:E-60116787-020-78565).

Results

Among the donor samples, 105 were obtained from living donors and 120 from deceased donors. Complement-dependent cytotoxicity crossmatch (CDCXM) was performed for all donor candidates in living donor transplants, while flow cytometry crossmatch (FCXM) was conducted only for the definitive donor. In contrast, all donor candidates in cadaveric transplants underwent FCXM. As a result, 39 FCXMs were performed in living donor transplants compared to 120 in cadaveric transplants (Tables 1 and 2). Repeated tests in living donor transplants were excluded from the analysis. No CDCXM T positivity was observed in living patients, while CDCXM B positivity was found in 5 (4.8%). In cadavers, CDCXM T positivity was found in 4 (3.3%), and CDCXM B positivity was found in 9 (7.5%).

FCXM results indicated that no FCXM T positivity was observed in living patients, while FCXM B positivity was found in 1 (2.6%). In cadavers, FCXM T positivity was found in 4 (3.3%), and FCXM B positivity was found in 10 (8.8%). In cadavers, T+B positivity was found in 4 (3.3%) in both CDCXM and FCXM. The findings in the living and cadavers are shown in Tables 1 and 2.

Table 1. Crossmatch test results in living donors

	CDCXM-T n (%)	CDCXM-B n (%)	FCXM-T n (%)	FCXM-B n (%)
Positive	0 (0)	5 (4.8)	0 (0)	1 (2.6)
Negative	105 (100)	100 (95.2)	39 (100)	38 (97.4)
Total	105 (100)	105 (100)	39 (100)	39 (100)

*CDCXM tests were performed for all donor candidates, and FCXM Tests were performed for Finalized candidates

Table 2. Crossmatch test results in cadaveric donors

	CDCXM-T n (%)	CDCXM-B n (%)	FCXM-T n (%)	FCXM-B n (%)	T-XM + B-XM n (%)*
Positive	4 (3.3)	9 (7.5)	4 (3.3)	10 (8.8)	4 (3.3)
Negative	116 (96.7)	111 (92.5)	116 (96.7)	110 (91.2)	116 (96.7)
Total	120 (100)	120 (100)	120 (100)	120 (100)	120 (100)

*T-XM + B-XM indicates patients who were positive for both CDCXM and FCXM. All patients with positive T-XM also had positive B-XM

Discussion

In this study, we evaluated pre-transplant lymphocyte crossmatch results obtained at the Pamukkale University Tissue Typing Laboratory between February 2020 and February 2024. Our findings demonstrated low positivity rates for both complement-dependent cytotoxicity crossmatch (CDCXM) and flow cytometric crossmatch (FCXM) among living-donor transplants, while slightly higher positivity was observed in cadaveric donor cases. This pattern is consistent with previous reports suggesting that cadaveric donors generally exhibit greater immunologic diversity and higher sensitization potential, leading to increased crossmatch reactivity [18-20].

The clinical relevance of crossmatch testing remains substantial, as the presence of donor-specific antibodies (DSAs) prior to transplantation is strongly associated with hyperacute rejection or early antibody-mediated rejection (ABMR). Therefore, crossmatch testing continues to be a cornerstone of immunological risk assessment in kidney transplantation [10, 21]. Despite advances in solid-phase assays such as Luminex single-antigen bead (SAB) testing, cellular crossmatch remains indispensable due to its ability to detect clinically significant complement-activating antibodies that may not be captured by solid-phase platforms [8].

The differences in analytical performance between CDCXM and FCXM have been well recognized. FCXM offers superior sensitivity by detecting low-titer IgG antibodies without requiring complement activation, thereby identifying DSAs that CDCXM may miss [21-23]. Nonetheless, whether this heightened sensitivity improves clinical outcomes is still debated. Several studies indicate that low-level DSAs detected solely by FCXM or Luminex are associated with a higher incidence of acute rejection but do not necessarily affect long-term graft survival when adequate immunosuppression is administered [24-26]. Thus, interpretation of FCXM results must consider antibody titers, HLA class specificities, and complement-binding capacity, as these parameters better reflect clinical pathogenicity [27].

The continuing role of CDCXM has also been questioned. Some centers advocate replacing CDCXM with FCXM and virtual crossmatch (vXM), given the rapid availability of donor HLA typing and recipient antibody profiles. However, CDCXM retains value, particularly in detecting strongly complement-binding antibodies and T-cell–positive reactions that correlate highly with hyperacute rejection risk [28]. The application of DTT in CDCXM helps eliminate false positives due to IgM antibodies, yet CDCXM remains limited by its complement dependency and inability to detect non–complement-binding IgG antibodies.

When compared with previous studies, the CDCXM-B positivity rate (4.8%) and FCXM-B positivity rate (2.6%) among living donors in our study were lower than those reported by Işıtmangil et al. [18], Shyti et al. [19], and Güngör [20]. These differences may stem from variations in sensitization levels within study populations, influenced by factors such as transfusion history, pregnancies, prior graft loss, and the prevalence of chronic inflammatory conditions. Methodological differences—including DTT usage, dilution strategies, and exclusion of repeated samples—may also contribute. Furthermore, higher crossmatch positivity observed among cadaveric donors in our study aligns with reports suggesting postmortem alterations in cell surface antigens, which may enhance nonspecific antibody binding [19].

This study has several limitations. As a single-center retrospective analysis, generalizability is limited. Repeated living-donor tests were excluded, potentially underestimating sensitization in certain subgroups. Additionally, we did not correlate pre-transplant DSA profiles or MFI values with post-transplant clinical outcomes such as acute rejection or graft survival. Future prospective and multicenter studies should examine the predictive value of CDCXM and FCXM in combination with Luminex-based DSA characteristics, including complement-binding assays (e.g., C1q, C3d) and dynamic changes during desensitization therapy [29].

In conclusion, the combined use of CDCXM and FCXM provides a robust and complementary approach to detecting donor-specific antibodies in the pre-transplant evaluation period. Our results support the continued use of both methods to improve immunologic risk stratification and enhance patient safety during donor selection.

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